

WHAT IS CLAIMED IS:

- 1 1. A method of inhibiting expression of an endogenous cellular gene
2 in a cell, the method comprising the step of:
3 contacting a first target site in the endogenous cellular gene with a first
4 zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;
5 thereby inhibiting expression of the endogenous cellular gene by at least
6 about 20%.
- 1 2. The method of claim 1, wherein the step of contacting further
2 comprises contacting a second target site in the endogenous cellular gene with a second
3 zinc finger protein.
- 1 3. The method of claim 2, wherein the first and second target sites are
2 adjacent.
- 1 4. The method of claim 3, wherein the first and second zinc finger
2 proteins are covalently linked.
- 1 5. The method of claim 1, wherein the first zinc finger protein is a
2 fusion protein comprising a regulatory domain.
- 1 6. The method of claim 5, wherein the first zinc finger protein is a
2 fusion protein comprising at least two regulatory domains.
- 1 7. The method of claim 2, wherein the first and second zinc finger
2 proteins are fusion proteins, each comprising a regulatory domain.
- 1 8. The method of claim 7, wherein the first and second zinc finger
2 protein are fusion proteins, each comprising at least two regulatory domains.
- 1 9. A method of inhibiting expression of an endogenous cellular gene
2 in a cell, the method comprising the step of:
3 contacting a target site in the endogenous cellular gene with a fusion zinc
4 finger protein comprising six fingers and a regulatory domain, wherein the K_d of the zinc
5 finger protein is less than about 25 nM;

0997844-070201

6 thereby inhibiting expression of the endogenous cellular gene by at least
7 about 20%.

1 10. The method of claim 1, wherein the cell is selected from the group
2 consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.

1 11. The method of claim 10, wherein the cell is a mammalian cell

1 12. The method of claim 11, wherein the cell is a human cell.

1 13. The method of claim 1, wherein expression of the endogenous
2 cellular gene is inhibited by at least about 75%-100%.

1 14. The method of claim 1, wherein the endogenous cellular gene is a
2 selected from the group consisting of VEGF, ER α , IGF-I, c-myc, c-myb, ICAM, and
3 Her2/Neu.

1 15. The method of claim 1, wherein the endogenous cellular gene is
2 VEGF.

1 16. The method of claim 1, wherein the inhibition of gene expression
2 prevents gene activation.

1 17. The method of claim 5 or 7, wherein the regulatory domain is
2 selected from the group consisting of a transcriptional repressor, an endonuclease, a
3 methyl transferase, and a histone deacetylase.

1 18. The method of claim 1, wherein the method further comprises the
2 step of first administering to the cell a delivery vehicle comprising the zinc finger protein,
3 wherein the delivery vehicle comprises a liposome or a membrane translocation
4 polypeptide.

1 19. The method of claim 1, wherein the zinc finger protein is encoded
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the
3 method further comprises the step of first administering the nucleic acid to the cell in a
4 lipid:nucleic acid complex or as naked nucleic acid.

0937644-070201

1 20. The method of claim 1, wherein the zinc finger protein is encoded
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a
3 promoter, and wherein the method further comprises the step of first administering the
4 expression vector to the cell.

1 21. The method of claim 20, wherein the expression vector is a viral
2 expression vector.

1 22. The method of claim 20, wherein the expression vector is a
2 retroviral expression vector, an adenoviral expression vector, a DNA plasmid expression
3 vector, or an AAV expression vector.

1 23. The method of claim 20, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to an inducible promoter.

1 24. The method of claim 20, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to a weak promoter.

1 25. The method of claim 1, wherein the cell comprises less than about
2 1.5×10^6 copies of the zinc finger protein.

1 26. The method of claim 1, wherein the target site is upstream of a
2 transcription initiation site of the endogenous cellular gene.

1 27. The method of claim 1, wherein the target site is adjacent to a
2 transcription initiation site of the endogenous cellular gene.

1 28. The method of claim 1, wherein the target site is adjacent to an
2 RNA polymerase pause site downstream of a transcription initiation site of the
3 endogenous cellular gene.

1 29. The method of claim 1, wherein the zinc finger protein comprises
2 an SP-1 backbone.

1 30. The method of claim 29, wherein the zinc finger protein comprises
2 a regulatory domain and is humanized.

1 31. A method of activating expression of an endogenous cellular gene,
2 the method comprising the step of:

3 contacting a first target site in the endogenous cellular gene with a first
4 zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;
5 thereby activating expression of the endogenous cellular gene to at least
6 about 150%.

1 32. The method of claim 31, wherein the step of contacting further
2 comprises contacting a second target site in the endogenous cellular gene with a second
3 zinc finger protein.

1 33. The method of claim 32, wherein the first and second target sites
2 are adjacent.

1 34. The method of claim 33, wherein the first and second zinc finger
2 proteins are covalently linked.

1 35. The method of claim 31, wherein the first zinc finger protein is a
2 fusion protein comprising a regulatory domain.

1 36. The method of claim 35, wherein the first zinc finger protein is a
2 fusion protein comprising at least two regulatory domains.

1 37. The method of claim 32, wherein the first and second zinc finger
2 proteins are fusion proteins, each comprising a regulatory domain.

1 38. The method of claim 37, wherein the first and the second zinc
2 finger protein are fusion proteins, each comprising at least two regulatory domains.

1 39. A method of activating expression of an endogenous cellular gene,
2 the method comprising the step of:

3 contacting a target site in the endogenous cellular gene with a fusion zinc
4 finger protein comprising six fingers and a regulatory domain, wherein the K_d of the zinc
5 finger protein is less than about 25 nM;
6 thereby activating expression of the endogenous cellular gene to at least
7 about 150%.

- 1 40. The method of claim 31, wherein the cell is selected from the
2 group consisting of an animal cell, a plant cell, a bacterial cell, a protozoal cell, or a
3 fungal cell.
- 1 41. The method of claim 40, wherein the cell is a mammalian cell.
- 1 42. The method of claim 41, wherein the cell is a human cell
- 1 43. The method of claim 31, wherein expression of the endogenous
2 cellular gene is activated to at least about 200-500%.
- 1 44. The method of claim 31, wherein the endogenous cellular gene is a
2 selected from the group consisting of FAD2-1, EPO, GM-CSF, GDNF, VEGF, and LDL-
3 R.
- 1 45. The method of claim 31, wherein the endogenous cellular gene is
2 VEGF.
- 1 46. The method of claim 31, wherein the activation of gene expression
2 prevents repression of gene expression.
- 1 47. The method of claim 35 or 37, wherein the regulatory domain is
2 selected from the group consisting of a transcriptional activator, or a histone
3 acetyltransferase.
- 1 48. The method of claim 31, wherein the method further comprises the
2 step of first administering to the cell a delivery vehicle comprising the zinc finger protein,
3 wherein the delivery vehicle comprises a liposome or a membrane translocation
4 polypeptide.
- 1 49. The method of claim 31, wherein the zinc finger protein is encoded
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the
3 method further comprises the step of first administering the nucleic acid to the cell in a
4 lipid:nucleic acid complex or as naked nucleic acid.
- 1 50. The method of claim 31, wherein the zinc finger protein is encoded
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a

FO2020-442680

3 promoter, and wherein the method further comprises the step of first administering the
4 expression vector to the cell.

1 51. The method of claim 50, wherein the expression vector is a viral
2 expression vector.

1 52. The method of claim 50, wherein the expression vector is a
2 retroviral expression vector, an adenoviral vector, a DNA plasmid vector, or an AAV
3 expression vector.

1 53. The method of claim 50, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to an inducible promoter.

1 54. The method of claim 50, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to a weak promoter.

1 55. The method of claim 31, wherein the cell comprises less than about
2 1.5×10^6 copies of the zinc finger protein.

1 56. The method of claim 31, wherein the target site is upstream of a
2 transcription initiation site of the endogenous cellular gene.

1 57. The method of claim 31, wherein the target site is adjacent to a
2 transcription initiation site of the endogenous cellular gene.

1 58. The method of claim 31, wherein the target site is adjacent to an
2 RNA polymerase pause site downstream of a transcription initiation site of the
3 endogenous cellular gene.

1 59. The method of claim 31, wherein the zinc finger protein comprises
2 an SP-1 backbone.

1 60. The method of claim 59, wherein the zinc finger protein comprises
2 a regulatory domain and is humanized.

1 61. A method of modulating expression of an endogenous cellular gene
2 in a cell, the method comprising the step of:

3 contacting a first target site in the endogenous cellular gene with a first
4 zinc finger protein;
5 thereby modulating expression of the endogenous cellular gene.

1 62. The method of claim 61, wherein the step of contacting further
2 comprises contacting a second target site in the endogenous cellular gene with a second
3 zinc finger protein.

1 63. The method of claim 62, wherein the first and second target sites
2 are adjacent.

1 64. The method of claim 63, wherein the first and second zinc finger
2 proteins are covalently linked.

1 65. The method of claim 61, wherein the first zinc finger protein is a
2 fusion protein comprising a regulatory domain.

1 66. The method of claim 65, wherein the first zinc finger protein is a
2 fusion protein comprising at least two regulatory domains.

1 67. The method of claim 62, wherein the first and second zinc finger
2 proteins are fusion proteins, each comprising a regulatory domain.

1 68. The method of claim 67, wherein the first and second zinc finger
2 protein are fusion proteins, each comprising at least two regulatory domains.

1 69. A method of modulating expression of an endogenous cellular gene
2 in a cell, the method comprising the step of:
3 contacting a target site in the endogenous cellular gene with a fusion zinc
4 finger protein comprising six fingers and a regulatory domain;
5 thereby modulating expression of the endogenous cellular gene.

1 70. The method of claim 61, wherein the cell is selected from the
2 group consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal
3 cell.

1 71. The method of claim 70, wherein the cell is a mammalian cell

1 72. The method of claim 71, wherein the cell is a human cell.

1 73. The method of claim 61, wherein the endogenous cellular gene is a
2 selected from the group consisting of VEGF, ER α , IGF-I, c-myc, c-myb, ICAM,
3 Her2/Neu, FAD2-1, EPO, GM-CSF, GDNF, and LDL-R.

1 74. The method of claim 61, wherein the endogenous cellular gene is
2 VEGF.

1 75. The method of claim 65 or 67, wherein the regulatory domain is
2 selected from the group consisting of a transcriptional repressor, a transcriptional
3 activator, an endonuclease, a methyl transferase, a histone acetyltransferase, and a histone
4 deacetylase.

1 76. The method of claim 61, wherein the method further comprises the
2 step of first administering to the cell a delivery vehicle comprising the zinc finger protein,
3 wherein the delivery vehicle comprises a liposome or a membrane translocation
4 polypeptide.

1 77. The method of claim 61, wherein the zinc finger protein is encoded
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the
3 method further comprises the step of first administering the nucleic acid to the cell in a
4 lipid:nucleic acid complex or as naked nucleic acid.

1 78. The method of claim 61, wherein the zinc finger protein is encoded
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a
3 promoter, and wherein the method further comprises the step of first administering the
4 expression vector to the cell.

1 79. The method of claim 78, wherein the expression vector is a viral
2 expression vector.

1 80. The method of claim 78, wherein the expression vector is a
2 retroviral expression vector, an adenoviral expression vector, a DNA plasmid expression
3 vector, or an AAV expression vector.

TO2020-4426550

1 81. The method of claim 78, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to an inducible promoter.

1 82. The method of claim 78, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to a weak promoter.

1 83. The method of claim 61, wherein the cell comprises less than about
2 1.5×10^6 copies of the zinc finger protein.

1 84. The method of claim 61, wherein the target site is upstream of a
2 transcription initiation site of the endogenous cellular gene.

1 85. The method of claim 61, wherein the target site is adjacent to a
2 transcription initiation site of the endogenous cellular gene.

1 86. The method of claim 61, wherein the target site is adjacent to an
2 RNA polymerase pause site downstream of a transcription initiation site of the
3 endogenous cellular gene.

1 87. The method of claim 61, wherein the zinc finger protein comprises
2 an SP-1 backbone.

1 88. The method of claim 88, wherein the zinc finger protein comprises
2 a regulatory domain and is humanized.

09897844-070204
T02020-44826860